

Original Article

Essential amino acid supplements increase muscle weight, bone mass and bone strength in adult osteoporotic rats

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Abstract

Protein undernutrition is known to play an important role in the pathogenesis of osteoporotic fracture in elderly. The mechanisms underlying the bone loss in protein undernutrition appeared to be related to an uncoupling between increased bone resorption and bone formation. This was associated with decreased plasma IGF-I levels, with anoestrus and decreased muscle mass. Reversibility of protein undernutrition-induced bone loss was investigated in ovariectomized adult rats, which were fed isocaloric 2.5 % casein diet (OVX2.5) for 16 weeks. Then, the animals were given a supplement of essential amino-acids in similar proportion to that of casein at doses of 2.5% (EAA2.5) or 5% (EAA5) of total food intake for an additional 16 weeks. Essential amino acid supplements increased bone mineral mass and strength in ovariectomized protein-deprived rats. EAA supplements were associated with stimulated bone formation and reduced bone resorption, with increment of plasma IGF-I and of limb muscle mass weight. These results suggest that nutritional intervention with essential amino acid supplements can increase bone mineral mass, bone strength and muscle mass in osteoporotic rats possibly by correcting IGF-I status.

Keywords: Amino Acid Supplements, Muscle Weight, Bone Mass, Bone Strength, Osteoporosis, Protein Undernutrition

Introduction

Protein under-nutrition is known to play an important role in the occurrence of hip fracture in elderly by increasing the propensity to fall as a result of muscle weakness and of impairment in movement coordination, and/or by decreasing bone mass.

A state of under-nutrition has been documented in elderly patients with hip fracture and in hospitalized elderly patients, low protein intakes were associated with reduced femoral neck bone mineral density (BMD) and poor physical performances. Thus, it appears that the integrity of the skeleton in elderly could be affected, not only by inadequate supply of bone mineral elements and vitamin D, but also by inappropriately low protein intakes.

In previous studies, we set up a model of selective protein under-nutrition in adult female rats. A decrease of bone mineral density was observed not only at the level of skeletal

sites containing trabecular bone but also at sites formed only by cortical bone. These modifications were observed with a protein under-nutrition obtained by feeding the animals a diet containing 2.5 % casein. Diet containing 5% casein and more did not effect bone mass. The mechanisms underlying the bone loss in protein under-nutrition were found to be an uncoupling between increased bone resorption and bone formation. This was related to a decreased plasma IGF-I and to anoestrus (both effects being additive on bone mass and strength). A decreased muscle mass was observed, which could also be involved in the maintenance of bone mass.

Since, isocaloric protein supplements attenuated bone loss, increased plasma IGF-I and positively influenced muscle strength in elderly post-menopausal women, we have investigated the effect of essential amino acid supplements in adult ovariectomized rats fed a low protein diet.

Methods

Reversibility of protein under-nutrition-induced bone loss was investigated in ovariectomized adult rats, which were fed isocaloric 2.5 % casein diet (OVX2.5) for 16 weeks. Then, the animals were given a supplement of essential amino-acids in similar proportion to that found in casein at

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doses of 2.5% (EAA2.5) or 5% (EAA5) of total diet for an additional 16 weeks. Control SHAM animal were pair fed a 15 % casein diet. In vivo bone mineral density (BMD) was measured by DXA using a Hologic QDR-1000 instrument adapted to measurement in small animals using an ultra-high-resolution mode. During the measurements, the animals were anesthetized with ketamine hydrochloride (100 mg/kg body weight). BMD, BMC and scanned area were recorded in vivo at the level of lumbar vertebrae, femur and tibia as previously described.

The in vivo reproducibility of these measurements, with repositioning, was below 1.8 %. The lumbar spine, tibia and femur were excised immediately after sacrifice. Lower limb muscle mass was evaluated. Ultimate strength (US) was evaluated at the level of lumbar spine, proximal tibia (PT) and midshaft tibia.

In each bone, the mechanical resistance to failure was tested using a servo-controlled electro-mechanical system (Instron 1114, Instron Corp., High Wycombe, England) with the actuator displaced at 2 mm/min. Both displacement and load were recorded. Ultimate strength (maximal load, N) and stiffness (slope of the linear part of the curve, representing the elastic deformation, N/cm) were calculated.

Reproducibility between vertebrae L3 and L4, between right and left tibia, and between femoral neck right and left, evaluated as the mean of coefficient of variation pairs, was below 4.8 %. Biochemical markers of bone remodeling (plasma osteocalcin, P Oc; urinary deoxypyridinoline, U dPyr) and plasma IGF-I were also determined. Osteocalcin and IGF-I were measured by radioimmunoassay, with reagents from Biomedical Technologies for the former, and with a kit from Nichols Institute after extraction by acid-ethanol and cryoprecipitation, for the latter. Total urinary deoxy-pyridinoline were measured after acid hydrolysis using a kit from Metra.

Results and Discussion

Essential amino acid supplements increased bone mineral mass at the level of skeletal sites formed by trabecular and cortical bone (proximal tibia and lumbar spine) as well as at the level of long bone diaphysis formed only by cortical bone (midshaft tibia and femur) in ovariectomized and protein-deprived rats. These effects on bone mineral mass were associated with positive effect on bone strength.

The results consisted in a real improvement of bone strength when compared to pretreatment value, at the level of vertebrae and proximal tibia, and by a prevention of further bone deterioration at the level of cortical area. As indicated by biochemical markers of bone remodeling, the mechanism underlying this marked effect on mechanical properties of essential amino acid supplements consist in a stimulation of bone formation and decrease in bone resorption. These effects on bone formation might be

related to the increment of plasma IGF-I. The increment of lower limb muscle mass could also be implicated by increasing mechanical stimulation of the skeletal pieces.

Conclusion

Essential amino acid supplements increased bone mineral mass and strength in osteoporotic rats. The isocaloric supplements increased plasma IGF-I above the levels of SHAM. As indicated by biochemical markers of bone remodeling, EAA supplements were associated with stimulated bone formation and reduced bone resorption. EAA supplements increased muscle weight.

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